

Amendments to the Specification

Please insert the Sequence Listing filed concurrently herewith.

Please replace the Title of the Invention at page 1, line 1, with the following amended title:

*Changed
from
old*
**INDUCTION OF CYTOTOXIC T LYMPHOCYTE RESPONSES USING
ANTI-CD40 ANTIBODIES**

Please replace the paragraph at page 9, lines 10-15, with the following amended paragraph:

B1
These antibodies and the method of making them are described in U.S. Patent No. 5,534,254 (Creative Biomolecules, Inc.). Different embodiments of bispecific antibodies described in the patent include linking single chain Fv with peptide couplers, including Ser-Cys, (Gly)₄-Cys (SEQ ID NO: 1), (His)₆-(Gly)₄-Cys (SEQ ID NO: 2), chelating agents, and chemical or disulfide couplings including bismaleimido-hexane and bismaleimidocaproyl.

Please replace the paragraph at page 12, lines 2-11, with the following amended paragraph:

B2
The EBV-transformed B-cell line JY and the myeloid derived cell line THP1 were cultured in T75 culture flasks routinely in Iscove's modified Dulbecco's medium (IMDM) to which 50 [☐\mug/ml gentamycin and 2% heat inactivated foetal calf serum was added ([☐FCS] FCS; BioWitthaker, Verviers, Belgium). The cells were cultured in a humidified incubator at 37°C and 5% CO₂. Once or twice per week the cells were split (1/20 to 1/100). To store the cell line, ampoules were made containing 5-10 x 10⁶

B2 Cont
cells/ml Hank's balanced salt solution (HBSS) supplemented with 20% [[FCSi]] FCS and 10% DMSO, and stored in [[the]] liquid nitrogen.

Please replace the paragraph at page 15, lines 11-18, with the following amended paragraph:

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B3
3 x 10⁶ THP-1 cells were first cultured for two days in 10 ml of IMDM + 2% of human type AB serum in the presence of 5 x 10² U/ml IFN-[[[]]] γ . Next the IFN-[[g]] γ treated THP-1 cells were washed once in IMDM + 2% human type AB serum. 10⁴ THP-1 cells per well per 96 [[w]] well plate [[well]] were cultured for two days in 120 μ l of culture medium diluted 1:2 with hybridoma supernatant. As controls CD154-mCD8 was used at 40 [[[]]] μ g/ml maximum and 2x dilutions and LPS at 20 ng/ml maximum and 2x dilutions.

Please replace the paragraph at page 15, line 20, to page 16, line 10, with the following amended paragraph:

B4
ELISA plates were coated with mouse anti human IL-8 antibody (Serotec) at 5 μ g/ml, 100 [[[]]] μ l/well for 2 hrs at room temperature on a plate shaker. The plates were then incubated with 1% BLOTTO for one hour on the plate shaker at room temperature. After four washings with PBS/Tween, 80 μ l of supernatants harvested from the THP-1 plate were added to the ELISA plate. For the IL-8 standards[[:]], IL-8 was diluted with 1% BLOTTO to 1000 pg/ml, 300 pg/ml, 100 pg/ml, 30 pg/ml, 10 pg/ml, 3 pg/ml, and 1 pg/ml. The ELISA plates were incubated for one [[hr]] hour at room temperature on the plate shaker. After four washings with PBS/Tween, 100 μ l/well mouse-anti IL-8 biotin conjugate (Serotec) was added at 1:1000 dilution in 1% BLOTTO and the plates were incubated for one hour at room temperature. After four washings with PBS/Tween, 100 [[[]]] μ l/well AMDX SA-HRP at 1:1000 dilution in 1% BLOTTO was added to the wells and the plates were incubated for 1 hour at room temperature on the plate shaker. After 4 washings with PBS/Tween, 100 [[[]]] μ l of TMB substrate was added to each well and the

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plates were incubated for 30 minutes at room temperature on the plate shaker. The reaction was stopped by addition of 50 μ l/well of 0.2 M H₂SO₄ and the plates were read with an ELISA reader at 450/590 nm.

Please replace the paragraph at page 18, line 26, to page 19, line 8, with the following amended paragraph:

b5

To screen for antibodies with agonistic activity, the selected supernatants containing CD40 binding antibodies were subsequently tested for their ability to induce IL-8 production in the CD40 expressing monocytic cell line THP-1, which had been pre-incubated with IFN- γ . ~~As shown in table 1, most~~ Most of the supernatants tested contained anti-CD40 antibodies[[,]] which displayed agonistic activity in this assay. Supernatants were arbitrarily subdivided into four different groups on the basis of their performance in the THP-1 assay (strong agonists with an OD of >2.000, intermediate agonists with an OD between 1.000-2.000, low agonists with an OD between 0.375-0.999 and non-agonists with an OD <0.375).

Please replace the paragraph at page 22, lines 5-24, with the following amended paragraph:

b6

Anti-CD40 antibodies that synergize with cCD40L in the induction of CD40 mediated activation of DC most likely show co-binding with sCD40L to CD40 and thus do not display strong blocking of binding of sCD40L to CD40. To screen for such antibodies, the percentage of inhibition of sCD40L binding to CD40 on JY EBV transformed B cells by the monoclonal antibodies was tested. This analysis revealed that there was strong variation in the degree that the monoclonal antibodies could inhibit the binding of sCD40L to CD40. Some antibody samples almost completely inhibited sCD40L binding, whereas other antibody samples could only partially block sCD40L binding or had no effect at all (~~table 2~~). The results were confirmed in the reverse way for a limited number of clones by testing the inhibition caused by the anti-CD40 monoclonal antibodies of the binding of CD40-Fc to CD40L expressed on the membrane

B6
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of PMA + ionomycin activated CD4+ T cells. In this experiment clone 4 blocked binding of CD40-Fc to CD40L on the T cells for 88%, clone 7 and 64 for respectively 16% and 25%. Although there was no absolute correlation between the performance of the antibodies in the DC maturation and the THP-1 assay and their ability to block binding to CD40, all the clones that did not block this interaction were non-responders in both assays (data not shown).

N.E.

Abstract

Please replace the Abstract with the amended Abstract provided herewith on a separate page.